Working through the bugs:

Application of gut microbiome analysis to prawn aquaculture to improve productivity

Agri-Science Queensland Innovation Opportunity

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Summary

The importance of the gut microbiome to health and wellbeing of humans and livestock is well understood, but this topic has only recently gained attention in aquaculture, particularly in prawn farming.

This project reviewed the current knowledge of the gut microbiome of farmed prawns and visited farms to establish how work in this area may benefit the local industry.

The review of recent literature found that:

- recent studies suggest that the gut microbiome probably plays as vital a role in farmed prawns as it does in other animals, with evidence emerging that a ‘normal’ microbiome exists.
- the prawn gut microbiome is however influenced both by feed and the environment- it is seeded by bacteria ingested with food and pond water.

Consultation with Queensland prawn farmers found that:

- there is widespread acceptance of the likely importance of the gut microbiome
- pond management strategies probably affect the bacterial microbiome of ponds, though the impact on the gut microbiome is unknown.

There is an opportunity to develop a collaborative RD&E project focussed on improving productivity of prawn farms using practices that manipulate the gut microbiome.

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Background

Higher animals, including vertebrates and invertebrates, host microbial life forms internally and externally, which are collectively referred to as the animal's microbiome. Different zones of the host animal typically support a characteristic microbial community, for example, the external surface and gut microbiomes are very distinct. Research of the gut microbiome has focussed predominantly on the bacterial component as it is considered to be the group most significantly contributing to host physiology and function.

It is now widely accepted that the microbiome of the gut is a critical part of the overall functioning of higher organisms. It has been shown for humans and a range of animals that microbiota inhabiting the digestive tract directly and indirectly fulfil critical roles in the breakdown of food, nutrient assimilation, host immunity and even host behaviour. Depending on the characteristics of the gut microbiome it can contribute to host health promotion and normal function (a state of symbiosis), or be detrimental (a state of dysbiosis).

The critical nature of the gut microbiome to animal function has been known for many years, but it is only over the last decade that its full interaction with the host beyond directly enhancing host digestive capacity began to be revealed in detail. Research is continuing to illuminate the pervasive role the human and livestock gut microbiome has on many aspects of physiology and behaviour.

Over the last three years DNA technology has been increasingly directed to understanding the microbiome of cultured prawns due to their great economic significance to many tropical and sub-tropical countries. As there are yet comparatively few prawn microbiome studies accumulated it is expected that in the coming years the true significance of the prawn microbiome to production efficiency will continue to be revealed as it has been for humans and other production animals.

Industry support for an investigation into the potential benefits of understanding and controlling the microbiome of prawns is critical as this R&D direction would only be pursued if the Australian prawn farming industry saw significant benefit of potential outcomes, such as improved productivity or lower production costs. As part of this project prawn farm operators were consulted to gain an industry perspective on the potential for pursuing the bacterial microbiome investigation.

Project Objectives

1. Complete a review of the current scientific understanding of the cultured prawn microbiome.
2. Survey prawn farm operators from across Queensland regarding:
    a. Their recognition of the potential importance of the bacterial microbiome to prawn and culture pond performance
    b. Farm outcomes and operator observations that may indicate potential microbiome-related influences
    c. Farm management practises that may directly influence the pond microbiome
    d. What they consider to be a good direction for any future microbiome investigation to take.
3. Produce a report that combines the outcomes of the above two objectives into a single document that will be a useful resource in the development of a robust R&D project to investigate increasing prawn farm productivity and sustainability through understanding and controlling the prawn microbiome.

4. Continue engagement with the prawn farming industry beyond the period of this ASQ Innovation project on the topic of prawn microbiome through extension materials including a poster for display at the Annual Australian Prawn Farmers Association (APFA) Symposium in August 2018, supply of the project report to the APFA and farm operators, and direct communication with individual farms. The objective is to work towards an expansive multi-agency R&D project.

Methodology

A literature review was conducted using online searching of scientific report databases to obtain information regarding the prawn microbiome, particularly cultured prawns; the black tiger prawn *Penaeus monodon,* and the Pacific white shrimp *Penaeus vannamei*. The review was written with industry operators as a primary intended audience and focussed specifically on the microbiome of prawns so that cited materials were directly relevant to their circumstances. It is considered that farm operators already have a background understanding of the microbiome concept, due at least in part, to the regular coverage of the human microbiome in mainstream media.

A two page summary of the literature review was sent to farmers prior to their participation in the farm survey to introduce the topic and stimulate their consideration.

Farms were visited in diverse areas of the state as the opportunity arose (Table 1). Additional farms will be visited in the near future as part of other activities. A questionnaire (Appendix C) was developed to guide the conversation around the status quo of the farms visited, their perspective on what's driving variability in pond outcomes and how their future plans might intersect with microbiome research.

Table 1. Prawn farms visited

North Queensland	Bundaberg region	Gold Coast region
Gold Coast Marine Aquaculture (North) Melivan P/L Truloffs (North & Gold Coast) Seafarms (3 farms)	Sunrise Seafoods Australian Coral Coast Mariculture Bundaberg Prawn Farm	Gold Coast Marine Aquaculture D&S Farms

Results

<u>Literature review</u>

A literature review entitled, “Application of gut microbiome analysis to prawn aquaculture to improve productivity” was produced (Appendix A).

Rate of publication on the microbiome of prawn aquaculture has increased in recent years. Currently there is convincing scientific evidence that the gut microbiome of prawns can exhibit wide variability and that its composition and structure can correlate with measures of immune function and stress. There is therefore a strong argument that pursuing the gut microbiome of prawns as an R&D direction could yield information that would contribute to real prawn farming industry benefits. The challenge will be to turn an enhanced knowledge of prawn gut microbiome dynamics into commercial benefit as practical tools will be required to control pond bacteriology at a commercial scale. Farms already exercise some microbial manipulation but it has not yet been demonstrated that these methods efficiently control the gut microbiome.

<u>Farm survey</u>

An outline of the survey findings is included in Appendix B.

The gut microbiome, the bacterial flora of the alimentary tract, is proving to be an important element in the health and resilience of all animals. Popular science media has been getting this important message out about its role in human health. Evidence indicates that this is likely also the case for farmed prawns – and there is no shortage of acute and chronic prawn health concerns. More and more research in this area is appearing. We’ve begun visiting prawn farms to get a better perspective of how research in this area might benefit the prawn aquaculture industry. Prawn farmers interviewed so far understand that gut microbiomes probably play an important role in their production systems, but they rightly want to know whether understanding it would improve outcomes on the farm. What can farmers realistically do about it? Fortunately, prawn farmers already have tools at hand that potentially change the pond and gut microbiome (e.g. molasses or probiotics), so scoping the potential to look more closely at this broad issue seems quite feasible. The overarching aim would be to help promote survival and growth.

Conclusions/Significance/Recommendations

We recommend a collaborative proposal be developed in consultation with industry and key collaborators to confirm the vital contribution of favourable microbiomes through experimentation. This work would seek to demonstrate their efficacy in supporting outcomes in tank and pond-scale production. It was noted during the farm visits that there is understandable scepticism about new products, equipment and processes promising great benefits. The communication/ extension strategy for the proposal will benefit from presenting a clear cost-benefit case to farmers and gathering feedback about the uptake of recent prawn farm management tools and innovations. The following are specific recommendations regarding RD&E activities to progress the topic:

1. Benchmark pond data across a number of prawn farms to confirm what is left to explain about pond outcome variation and then demonstrate that a significant change could be detected by a realistic farm-scale microbiome-focused study.
2. Develop a proposal looking at the use of microbiome control in improving pond production. By including the use of off-the-shelf products, further external funding may be drawn to the work.

3. Prepare a communications/extension strategy for the study that builds on best-extension practice. Establish how recent farm innovations have entered into use by consulting widely with farmers, project partners and extension experts.

Key Messages

- While understanding of the gut microbiome of farmed prawns and its significance to productivity is limited, recent papers suggest that the variability observed can correlate to measures of immune function and stress.
- Prawn farms in Australia can experience significant production losses due to bacterial and viral diseases. While pathogen-free post-larvae can be stocked, bolstering the resilience of the crop may help protect against pathogens entering horizontally, via seawater exchange.
- Prawn farmers are already interested enough to make scoping a proposal worthwhile- as long as practical outcome can be demonstrated.
- Tools already exist that affect the pond microbiome, and current technology allows us to measure changes in the gut microbiome with a high degree of accuracy.

Where to next

The next step is to develop a project outline and take the project proposal to farmers and potential collaborators. The latter includes CSIRO, James Cook University and feed manufacturers.

Further discussion with the prawn farming industry and potential collaborators will be undertaken at the Australian Prawn Farmers Association Annual Symposium in August 2018. A poster will be produced for display at this event and the project report will be refined and used as a resource for framing discussions.

Budget Summary

The project was allocated $2000 for operating costs. $1458.41 of this budget was used for travel to prawn farms along the Queensland coast by Dr Brian Paterson to interview the farm manager/operator and other staff and complete the microbiome survey.

Appendix A. Literature review.

Application of gut microbiome analysis to prawn aquaculture to improve productivity.

Introduction to the gut microbiome

Higher animals, including vertebrates and invertebrates, host microbial lifeforms internally and externally, which are collectively referred to as the animal's microbiome. Of the organisms present, prawn farmers will be familiar with bacteria and fungi but the constituents include less familiar bacterial-like taxa called archaea, and a grab-bag of nucleated unicells or protists that don't fit in the usual categories. Different zones of the host animal typically support a characteristic microbial community. For example, the microbiome of the external surface is distinct from that of the gut. Research on the gut microbiome has focused predominantly on the bacterial component as it is considered to be the group most significantly contributing to host physiology and function.

It is now widely accepted that the microbiome of the gut is a critical part of the overall functioning of higher organisms. It has been shown for humans and a range of animals that microbiota inhabiting the digestive tract directly and indirectly fulfil a critical role in the breakdown of food, nutrient assimilation, host immunity and even host behavior. Depending on the characteristics of the gut microbiome it can contribute to host health promotion and normal function (a state of symbiosis), or be detrimental (a state sometimes called dysbiosis).

The critical nature of the gut microbiome to animal function has been known for many years, but it is only over the last decade that its full interaction with the host beyond directly enhancing host digestive capacity began to be revealed in detail. Research is continuing to illuminate the pervasive role the human and livestock gut microbiome has on many aspects of our physiology and behavior.

Over the last three years DNA technology has been increasingly directed to understanding the microbiome of cultured prawns due to their great economic significance to many tropical and sub-tropical countries. As there are yet comparatively few prawn studies accumulated to date it is expected that in the coming years the true significance of the prawn microbiome to production efficiency will grow, much as it already has for humans and livestock. The discussion of the gut microbiome that follows focuses on the current understanding of the penaeid prawn gut microbiome for direct applicability to the Australian prawn farming industry. Questions addressed include: Can a greater understanding of the cultured prawn gut microbiome be used to enhance pond production predictability and increase total production of prawn farms, and, is it possible for farms to manipulate the gut microbiome of pond cultured prawns to promote health, growth and disease resistance?

Prawn gut microbiome

The bacteria found in the prawn alimentary system is collectively, the gut microbiome. Testing reveals a wide and volatile diversity of taxa. Analysis of the prawn gut microbiome using DNA sequencing techniques reveals a high level of detail regarding its composition, providing identification of taxa from high level groupings, such as phyla and families, through to the species level. Recent studies indicate a relatively rich diversity of bacteria in the guts of cultured prawns, with around a dozen phyla compromising many genera and in excess of 100 species idedntified in the guts of farmed prawns (Chaiyapechara et al., 2012 ; Huang et al., 2016). With potential for large numbers of bacterial species present in the prawn gut, there is also potential for meaningful variability in community structure, both as presence or absence of certain types and their relative abundance.

There is also a marked difference in gut bacterial community structure being found among cultured prawns from different farms (Chaiyapechara et al., 2012). The gut microbiome can be an indicator of animal stress (Llewellyn et al., 2014), and prolonged stress is known to be detrimental to immune and other physiological function (Direkbusarakom & Danayadol, 1998 ; Tseng & Chen, 2004 ; Sanchez et al., 2001 ; Wang & Chen, 2006), which in turn has ramifications for health, survival and growth. The 'normal' microbiome shows through this variability: there appears to be core species that persist despite environmental variability and ingestion of different feeds (Huang et al., 2016 ; Rungrassamee et al., 2014). Displacement of this normal group of bacteria by other species may indicate stress (Cornejo-Granados et al., 2017 ; Xiong et al., 2017), and disease is expected when pathogens proliferate there. For *P. vannamei*, evidence supports gut microbiome being a reliable indicator of a prawn susceptibility to disease and the amount of gut microbiome deviation from normal is correlated with severity of disease (Xiong et al., 2015 ; Xiong et al., 2017).

What explains the changeable community structure? The microbiome of the digestive system persists by using ingested material as a substrate for growth. In normal healthy prawns bacteria present in the stomach originate from ingested food with little, if any, direct colonisation of the internal gut lining (Soonthornchai et al., 2015b). Many bacteria harmlessly transit the gut within the ingested food, but some persist for longer for good or ill by attaching to the gut lining.

The food peritrophic membrane is a vital but not inviolable barrier to bacterial infiltration of the gut wall (Peters, 2012 ; Soonthornchai et al., 2015b). The gut of prawns is divided into three main sections: foregut, midgut and hindgut. The foregut, (the oesophagus and stomach) and hindgut are lined with chitinous cuticle that is renewed with each moult, but the midgut and the associated 'midgut gland' or hepatopancreas is protected with this peritrophic membrane (Felgenhauer, 1992). This membrane wraps the cord of digested food leaving the stomach, separating this foreign matter from the lining of the midgut and hindgut (Wang et al., 2012 ; Martin et al., 2006). Yet bacteria colonise these surfaces, and some serious pathogens, for example *Vibrio parahaemolyticus*, can compromise the integrity of the peritrophic membrane.

<u>Origins of the prawn gut microbiome</u>

The gut microbiome is influenced by factors in the host's environment as well as internal factors specific to the type of host (Li et al., 2018).

Feed and other ingested material brings bacterial inoculants and substrates (Soonthornchai et al., 2015a). Some substrates may favour some classes of bacteria over others (Huang et al., 2016). Penaeid prawns subjected to starvation stress also show distinctive changes to the gut microbiome that correlate with reduction of both digestive capacity and immune system function (Dai et al., 2018). These points show that feeding rates and management can have a direct effect on the prawns gut microbiome.

Aquatic animals also drink, so the aquatic microbiome and other characteristics of the pond water can impact the gut biota. Problems may be compounded by poor water quality. Sulphide is often present in pond sediments as hydrogen sulphide, and affects the gut microbiome of *P. vannamei* in a dose dependent manner, promoting higher levels of potentially pathogenic bacterial groups (Suo et al., 2017).

Nevertheless, an animal's insides can differ in specific ways from conditions outside. While transient bacteria are not strictly inside the prawn, the typical physiological conditions found at the gut lining in part explains the persistence of a 'normal' microbiome typical of the animal (Colston & Jackson, 2016 ; Huang et al., 2016).

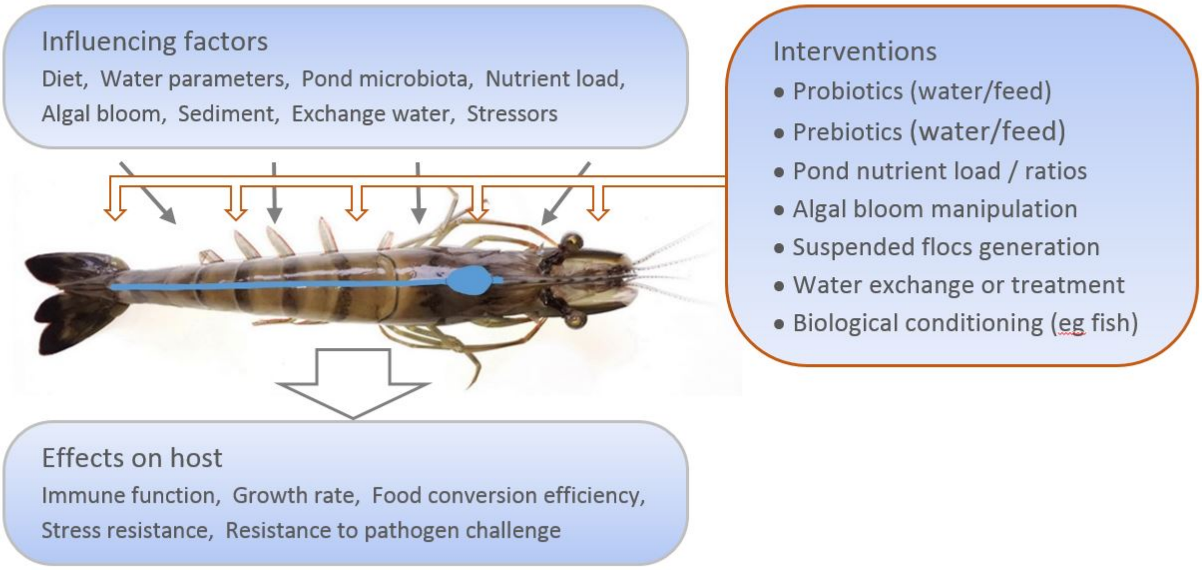

Figure 1. Diagrammatic summary of the main aspects related to the gut microbiome of farmed prawns.

Manipulating the gut microbiome of cultured prawns

The gut microbiome may be manipulated indirectly via the pond environment or directly via ingested food. However, the interaction between the pond and the gut microbiome is not well explored. Nutrient accumulation drives the pond ecosystem and managing the passage of photosynthetic microalgae blooms may alter unseen components of the pond microbiome. Microalgae strongly influence the bacterial community through extracellular bioactive compounds (Rehnstam-Holm & al., 2010) (Dash et al., 2017). Manipulation of the physico-chemical environment of the pond may also be an option but there is scant information in the literature on the interaction between underlying environmental variables and the pond microbiome. Temperature and salinity of ponds will influence their bacteriology but controls on these variables may be impractical. Anecdotal evidence from farm tests in Asia identified that the bacterium responsible for Early Mortality Syndrome was influenced by water pH within the practical range of 7.5 to 8.5 (Chamberlain, 2013). To a certain degree, farms in Australia can manipulate pH through addition of carbonates, managing the phytoplankton bloom, or addition of molasses (Smith & West, 2011).

It is not yet clear what effect reduced sediments and low oxygen zones in the pond have on the prawn gut microbiome. Dissolved oxygen levels exert a strong influence on pond microbiology, and this is best demonstrated in the bottom sediments which exhibit an extreme range of levels of anoxia with discrete zones characterized by different bacterial assemblages (Robinson et al., 2016). The detrimental impact of hydrogen sulphide on gut microbiome is important in this regard as mentioned above.

Some studies already show that probiotics potentially influence the gut microbiome of prawns and may provide a pathway to improved health status and growth. Probiotics in prawn farming were first promoted to exert control on the pond microbiome, to process accumulating waste more efficiently and exclude certain pathogenic bacteria from the water-column (Ninawe & Selvin, 2009). A range of probiotic products are marketed to aquaculture producers, and some are promoted as products specifically for prawn aquaculture. These bacteria are unlikely to belong to the prawn's 'normal' gut microbiome and uptake of the technology is not widespread. Yet, manufacturers claim benefits ranging from greatly reduced organic waste accumulation in the pond, through to healthier, faster growing prawns with higher resistance to disease challenge. Not unexpectedly, recent research confirms that adding probiotics to prawn production systems can modify the gut microbiome (Kongnum & Hongpattarakere, 2012 ; Vargas-Albores et al., 2017). Probiotics have recently been reported to confer a measurable benefit to prawn immune function, stress resistance and food conversion ratio (Olmos et al., 2011 ; Rengpipat et al., 2000 ; Zhang et al., 2010).

Probiotics are also fed to some farmed fish and other livestock (Hai, 2015). Another emerging tactic, akin to adding molasses to ponds, is to investigate fortifying feed with compounds called prebiotics that favour growth of desirable gut bacteria. Compounds investigated for this purpose include immunostimulants, vitamins and nucleotides (Ghanbari et al., 2015). In this context, studies should also investigate the impact on prawn gut microflora by the prawn feed additive Novacq^{TM}, which is the residue of a marine pond microbiome.

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Appendix B. Farm visits

Background

The recent appearance of PMMS in the hepatopancreas or midgut gland of prawns in some prawn farms in the Bundaberg region was a timely reminder that gut bacterial microflora is the often overlooked first line of host-defense in many organisms. Investment in domestication and tighter hatchery biosecurity are expected to reduce vertical transmission of pathogens from broodstock into farms. But pathogens can still enter farms horizontally from coastal waters and prawn farmers certainly cannot control the weather. Prawns farmed in outdoor systems will therefore become stressed from time to time, and classically stress reduces an organism's resilience in the face of pathogen incursions. But farmers can intervene in the host-pathogen-environment nexus though what they feed their prawns and how they manage the pond water.

Hence the idea to visit prawn farms and get farmers perspective on the question of the prawn microbiome. One hypothesis we tested was that there is unexplained variation in pond production that we aren't capturing amongst the usual suspects: ponds behaving badly for no known reason. That might have been evidence of 'bad' microbiome that doesn't express through the usual pathogens. We didn't find widespread confirmation for this (encouragingly), instead these preliminary results suggest such troubles may be site or locality linked. In this report, a way forward is proposed as a result of these discussions.

Methods

Farms were visited in diverse areas of the state as the opportunity arose. Other farms will be visited in the near future as part of other activities. The questionnaire (Appendix C) was developed to guide the conversation around the status quo of the farms visited, their perspective on what's driving variability in pond outcomes, and how their future plans might intersect with microbiome research.

Results

Prawn gut microbiome.

Farmers interviewed understood the principles behind gut microbiome and its role in animal health by analogy with the attention paid to this factor in popular science and health media. It's also a concept with some standing in prawn aquaculture, for example microbial hygiene is an important facet in hatchery operation: probiotics are routinely used to control 'bad' bacteria in batch cultures. Further, the bacterial vectors of “Early Mortality Syndrome" overseas, like that of PMMS in Australia, are various species/strains of marine *Vibrio* bacteria that acquire a toxin gene (Pir A/B) that causes invasive gut pathology. We also have to be realistic here. Talk of correcting the microbiome reminds farmers that when running ponds they are managing a large complex biome of sometimes difficult-to-control micro-plankton blooms. Their immediate response is, is this other 'biome' a real problem and what can they do about it? Well, ultimately intervening in a prawn farm this way has to be about feeding and growth. Showing a difference to the bottom line. Further conversation suggests there are already ways that farmers intervene with the microbiome, but the fact that they are not widely understood or specifically used indicates that an experiment itself is not enough - serious attention to the uptake of new methods needs to be given.

Farm sites and stocks farmed.

The farms involved in the survey ranged from the oldest farms in the state, started 30 years ago, to some the last farms constructed, just after the millennium. The age of ponds doesn't seem to be an overriding concern- but a small number of problematic ponds were mentioned (apparently due to soil or site-related issues). Most of the older farms started small and usually expanded in subsequent years, so many farm managers have experience with running newer ponds.

Of the eight companies visited, five have only one farm, one runs three farms in one region (Cardwell-Ingham) and two have northern and southern farms. One to four people participated in each survey. Staff from multiple-farm companies usually have experience working for previous owners or on other farms. Of the five single-farm companies, three were the first owner/managers of the farm (or took over from parents), and two were run by managers who have worked at other sites. Some comments were made about existing farm designs, placing certain ponds in unfavourable locations, and in some unorthodox pond designs (e.g. centre drains, very large ponds) which had to be corrected.

Today, the use of Northern Territory (NT) breeders is widespread, though east coast (EC) stock is still used. It's just over ten years since use of NT stock became common. East coast stock was the original resource but it was limited in availability. Farmers surveyed who rely on other hatcheries stated that they often cannot always be choosy in their post-larval supplies. NT stock is favoured because itperforms better in farms, but it is more expensive, the logistics are more difficult and suppliers come and go (it's a tendered contract). Inexperienced suppliers may not deliver the goods. For example this year, the NT females supplied were too immature, forcing some hatcheries to turn to EC stocks as a fall back measure.

Farm management issues

Most farmers visited nominated no inexplicable pond outcomes, i.e. no ponds performed unusually poorly or well without some apparent explanation. Generally, farmers felt ponds delivered not too far either side of an average for the season. There was usually an explanation if a pond disappointed. Two farms felt that around 10% of ponds were outliers for reasons unknown. That is enough ponds in the total industry to make one small farm of around 10 ha.

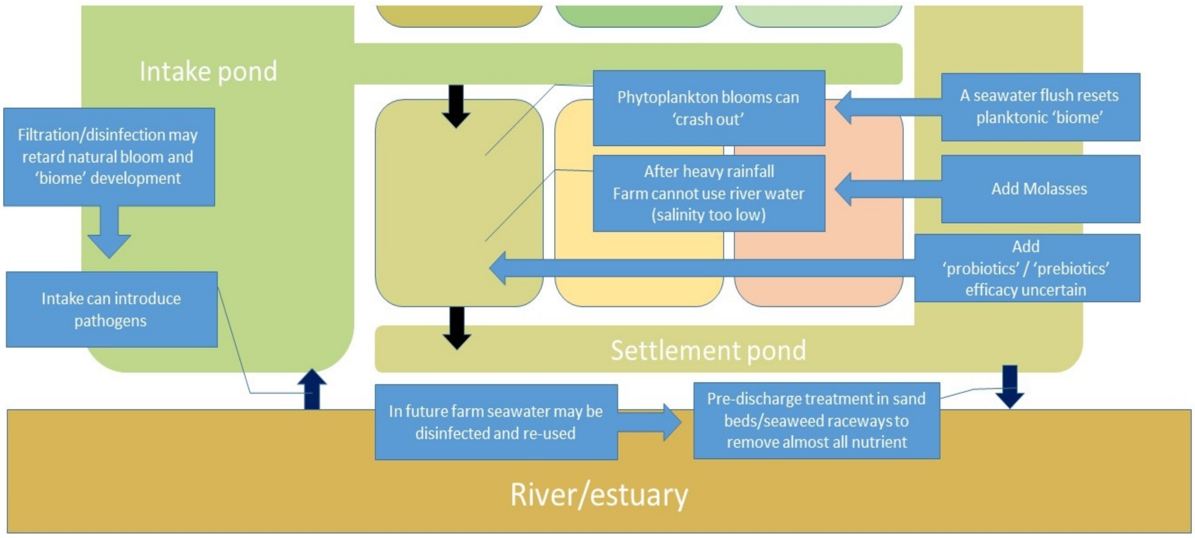

Figure 2. Diagrammatic summary of how pond management may impact the gut microbiome of farmed prawns.

Farmers commonly nominated post larval quality, harmful algae blooms and disease amongst the three most important causes of poor pond outcomes on a farm, though order varied from farm to farm. Weather and incoming water quality also figured amongst the top five, though these impact the entire farm, or even multiple farms along the coast. Farmers can call other farmers when prawns unexpectedly stop feeding- if anomalies are happening at several sites it may be a weather system or perhaps the brand of feed.

While harmful blooms or bloom management were commonly cited as possible issues by farmers, most farms only monitored algal blooms on the spot using secchi disks. Most farms take secchi readings (water transparency) at least once a day. Daily water quality measurements are mostly confined to parameters measured using popular multi-probe field instruments (e.g. DO and pH). Monitoring ammonia and alkalinity was less common- as you might expect for a less frequent issue.

Farmers typically use feeds from two companies and manufacturers market feeds on performance rather than 'health' *per se*. The reasons for choosing more than one supplier varied from farm to farm. Some larger farms ran in-house trials to compare the result. Some farms chose feeds to manage production costs- for example one farm chose more expensive feed knowing the prawns could be harvested sooner, to meet a market need. Imported feeds were generally cheaper, but sourcing them was admitted to be more onerous and they weren't as fresh. A few farms identified one imported brand that recently returned very unsatisfactory results.

Most farmers don't regularly use water quality interventions like molasses in ponds and there is a lot of scepticism about the likely benefits of probiotics. A couple of farms use molasses regularly to control water quality and the majority of farms have trialled it at some time.

<u>The future</u>

Many farms may have reached their maximum size but a couple of farms are expanding or have large new farms in the pipeline. Discharge regulations are not the insurmountable barrier to farm expansion they once were as farms are able to sufficiently manage effluent quality. Companies already farming in more than one location/region are the farmers expanding or in the process of building new farms. The last wave of farms constructed or expanded in Queensland already have settlement ponds on their discharge. Some producers had their stomach for expansion turned by environmental regulations and cheap seafood imports at the millennium, and the arrival of white spot disease (WSSV) has made others cautious. Owners of some early farms have meanwhile been ironing out idiosyncrasies in farm design (e.g. splitting overly-large ponds into smaller units).

The majority of farmers expect to remain focussed solely on prawn production for now but some are experimenting with fish such as Queensland groper and cobia. Farms are already licensed to grow numerous species but farmers generally don't think the figures for fish stack up, certainly nothing like those for prawns.

Farmers are noting developments with water quality instrumentation and auto-feeders but have other demands on their money for now. They see practical hurdles of cost and additional maintenance and labour.

Intake filtration is emerging as the new challenge, particularly for farms in the Gold Coast Region due to the threat posed by Moreton Bay's WSSV outbreak. A couple of farmers were interested in growing fish in intake reservoirs to condition seawater and blooms destined for prawn ponds.

Farmers in the Gold Coast Region accept that farm recirculation may be where things are ultimately heading- for biosecurity purposes. If you are cleaning the water- why throw it away? Some farms in the north are reusing pond water to some extent, for example when floods make exchange impossible, or to kick-start blooms in recalcitrant ponds. There is wide recognition that there are biosecurity risks with re-using seawater within the farm that need to be addressed.

Discussion

Farmers interviewed from diverse operations understood that the gut microbiome probably played a major role in the life of their prawns, but wanted to know what they could realistically do about it. Would understanding gut bacteria improve their ability to farm prawns? Popular science media has certainly got the message out about the ubiquity and hitherto uncelebrated role of ‘good' bugs inside ‘higher organisms’, and prawn farmers have no shortage of acute and chronic prawn health concerns. So the farmers are already interested enough to make scoping a proposal worthwhile. One of the major perils in prawn farming overseas, AHPND, is a gut bacterial disease- and this toxin has appeared in local *Vibrio* species most recently in some Bundaberg region farms. Since the gut microbiome can possibly go ‘bad’, our opening hypothesis to quantify the possible extent of the problem was to ask if a fraction of ponds might give poor growth for no known reason. The aim was to try to quantify what is being changed. As it turned out, the majority of farmers saw no inexplicable outliers in pond results. So where is the problem? While the microbiome may be very important an immediate focus is needed on the production data that we are trying to influence. A number of recommendations are presented here to go forward.

Recommendation 1: Benchmark pond data across a number of prawn farms to confirm what is left to explain about pond outcome variation, and then demonstrate that a significant change can be detected by any realistic design proposed for a farm-scale microbiome-focused study.

The techniques exist to monitor pond and gut microbiomes. JCU has initiated work in this area and DAF is working up methods to examine how new intake water treatment methods impact the microbiome of pond water. But producers will expect to see work in this area grounded in outcomes that they can measure- i.e. feed consumed, growth rate achieved and the harvested tonnage. In the near future the APFA can expect to reap the benefits of valuable work already in progress, like brood stock domestication and biosecurity improvement. Microbiome research is about what happens after that. Ponds receiving healthy PLs will still be vulnerable to adverse weather, to harmful blooms and to endemic pathogens entering from the immediately environment.

The variation that remains after removing hatchery biosecurity/PL issues will tell us two important things, firstly the role that outlier ponds might play in the data, and secondly, what kind of experimental design on a farm is needed to demonstrate a significant average improvement over the status quo. It may be important that prawns go “off” their feed when stressed and resume feeding when conditions improve. That apparently happens to most farms at some time or other. A good first step then would be to quantify this issue objectively using existing, de-identified-farm data. A hypothesis to explore in the experimental phase below would be whether feeding data tells farmers what the prawn gut microbiome is doing.

Recommendation 2. Develop a proposal looking at the use of microbiome control in improving pond production, and by including off-the-shelf products, further external funding may be drawn to the work.

Suggested objectives for this proposal are:

1. Confirming the role of the microbiome through experimentation (feeds, pre-biotics, and probiotics) and identify favourable biomes that confer production advantage.
2. Determine practical methods for microbiome manipulation through sub-commercial scale experimentation (tank-scale) before proving promising options in commercial ponds.

Comparing the microbiome of prawns in ponds that are growing well, to prawns in ponds that aren't growing, is a start- but producers already know the prawns aren't growing well in a 'poor' pond. Is the difference predictive, or are the results describing the microbiome of undernourished prawns? The idea would be to try to recreate these differences under controlled conditions.

After surveying what happens to the microbiome in ponds, the next step is to conduct laboratory feeding trials to find evidence of a microbiome effect on growth that would justify a farm-based growth trial. For example if the same feed is offered does regular addition of pre- or probiotics improve growth? How the proposed supplements square with regulatory requirements (e.g. APVMA) would also need to be addressed.

Evolution of farm design is a long-term strategy. Prawn farms are currently designed to control the pond microbiome using water renewal. Modifying the water treatment infrastructure of an existing farm is a high capital investment but it is happening due to the risks of horizontal transmission of exotic prawn pathogens from the sea into our southern-most farms. Farms elsewhere are also expanding and new farms while not yet built, are finding ways through the regulatory gauntlet. So discussions with stakeholders about the prawn farm microbiome proposal can consider experiments on sites where microbial 'capital' (e.g. bio-reactors) is built into the farm. Opportunities exist to investigate whether 'upstream' microbiomes, e.g. fish ponds, sand beds or seaweed raceways, can play a role in improving the health and growth of the crop.

Recommendation 3. Prepare a communications/extension strategy for the study that builds on best-extension practice. Establish how recent farm innovations have entered into use by consulting widely with farmers, project partners and extension experts.

Developing new recipes to dose into prawn ponds is not the final objective- these new methods must be accompanied by robust cost:benefit information that clearly states the business case to the industry. There is understandable scepticism about new products, equipment and processes promising great benefits. Farmers know that phytoplankton blooms are difficult enough to manage without widening the farmer's job to also controlling bacteria. Perhaps the nub of this issue is that probiotics are already marketed for prawn ponds, most local farms have tried them in some form or other, but few farmers use them today. Yet probiotics or additions of molasses is what farmers have in mind as a straight-forward intervention. Farmers know that molasses awakens dark pathways in an otherwise phototrophic pond: it recruits the *heterotrophic* microbiome. That step aims to control pond water quality when river floods prevent pond water renewal. Feedback about the roll-out of the molasses technique might be a good way to develop new extension strategies here.

Appendix C. Prawn farm questionnaire

Prawn farm microbiome Survey

This information is being collected to gauge the priority / feasibility of research specifically focusing upon improving prawn crop performance by manipulating the microbial flora or 'microbiome' of the prawn-gut and pond. Responses from individual farms will be made available only to those respondents. Results used to inform government and industry research direction will be in presented only in summarised/aggregated form from which individual responses cannot be identified.

Introduction- these initial questions attempt a snapshot of the participant's experience and draw out information about possible longer term changes in the farm environment

1. You been farming prawns for how many years? [ ]
2. What prawn stock are you farming now [ ] East Coast [ ] GoC [ ] NT
   Any observations on past/current performance of these stocks?
3. How old are the ponds in this farm (When was it built or expanded)?
4. Do crops in your ponds here sometimes give unexpected outcomes: poor or even good results that you cannot explain from experience? □ Yes continue 4a-c , □ No go to next question

4a Do you see crop that show performance above that of other ponds that cannot be explained?
[ ] Yes [ ] No.

4b Have you noticed any trends over time in variability of pond outcomes across the farm?

4c Is this farm your only prawn farm (include previous owned farms)? , □Yes, go to Present observations. □ No go to 4d

4d Have your other farms produced crops with unexpected outcomes at a similar rate or does the phenomenon differ elsewhere from your experience at this farm?

Present observations- these questions are about farmer's experience of problematic crops and collect data to estimate the cost of 'problem crops' to the industry – ponds with inexplicably poor outcomes.

If you answered yes to Question 4 – inexplicable poor outcomes- above...

5. What is the extent and prevalence of these under-performing ponds (proportion of ponds)?
6. Are some particular ponds on site consistently yet inexplicably underperforming?
7. How is the poor outcome expressed [ ] FCR, [ ] growth rate, [ ] survival, [ ] total production, [ ] product appearance, [ ] other..

8. What factors do you think might be driving this unusual crop variability?

9. If you have an unusual response in a pond (not a disease outbreak) who do you turn to assist you to understand the cause?

10. If you have investigated the cause of unusually poor crops. How did you do it? What if anything did you learn?

11. Do you ever see unusual or unexpected prawn behaviour in ponds that is not explained by obvious pond conditions, e.g. oxygen level?

The status quo: These questions address the microbiome's standing amid current farm management practices, factors known to cause poor crop outcomes, and data that farms collect.

12. Please rank the factors (1= most likely/frequently) that you believe explain known poor crop outcomes

[ ] PL Quality	[ ] pond / equipment faults	[ ] incoming water quality	[ ] disease/ pathogens	[ ] Harmful algae blooms
[..] old or new ponds	[..] time of year	[..] weather	[..] cyclones	[ ] feed quality

Comments: Include any other factors you know of.

13. What PL stocking density do you typically use (PL/m2)

[ ] < 30	[ ] 30-40	[ ] 40-50	[ ] >50

14. As a guide, what exchange rate per week do you use for ponds in each third of the production cycle

First Month	Second Month	Mid-crop	End-crop

15. What factors do you currently measure to track pond/crop performance? (?Frequency)

[ ] FCR (?)	[ ] DO (?)	[ ] pH(?)	[ ] Secchi (?)	[ ] PL qual
[ ] Other, for e.g.	[ ] ammonia	[ ] alkalinity	[ ] Algal bloom	

16. Do you also measure /record other information about the pond ecology or 'biome' – e.g. algal bloom, zooplankton.

17. Do you use more than one feed company's feed? Why?

18. Do feed manufacturers already make claims about feeds supplied having probiotic or prebiotic effects, promote 'good bacteria' or promote benefits to prawn 'health'

19. Do you apply 'probiotics' to the ponds or via feeds (e.g. alter the micro-biome)?

20. Do you add molasses to stimulate microbial-floc? (again, this is the your 'microbiome' at work). How successful is this?

Future intentions- these questions are about how the industry and the farming environment might evolve during the coming R&D cycles- which potentially alters the way a microbiome study would be undertaken.

21. Do you intend to modify/expand this farm? How soon?

22. Will your farm grow more species in future (What species are on the license now?)

23. Will you further manipulate the water coming onto the farm or leaving it (via settlement, filtration, and disinfection)? How soon?

24. Are you interested in recirculating the farm water – even if only on a temporary basis?

25. Are you interested in installing automatic feeding and or water quality monitoring equipment?

26. If not, what do see would be the obstacles to adoption of new feeding/monitoring technologies?

27. If you wanted ONE thing to better control crop performance what R&D direction would assist this?